

CONTRIBUTIONS FROM THE LABORATORY OF VERTEBRATE BIOLOGY

University of Michigan, Ann Arbor, Michigan

Number 59

February, 1953

THE MEASUREMENT OF FERTILITY AND ITS USE AS AN INDEX OF REPRODUCTIVE ISOLATION AMONG CERTAIN LABORATORY STOCKS OF PEROMYSCUS

By T. T. LIU

Accepted for publication September 15, 1952

CONTENTS

Introduction	1
Materials and Methods	2
Analysis of Data	6
Summary	11
Literature Cited	12

INTRODUCTION

MOST previous studies of hybridization have been restricted to its qualitative aspects. Quantitative measurements of the fertility relationships within and between species are urgently needed. To meet this need some simple formulae are here presented which have been derived in an attempt to evaluate the fertility of parent stocks as well as the amount of reproductive isolation which is involved in intersub-specific and interspecific crosses in *Peromyscus*.

Mice of the genus *Peromyscus* offer a unique opportunity for the study of hybridization. They occupy a variety of ecologic niches and are divided into many species and subspecies. Most species can be bred in the laboratory, and some interspecific and intersubspecific crosses are successful (Dice, 1933).

Acknowledgments

I wish to express my sincere appreciation to Dr. J. V. Neel for his valuable advice and guidance during the progress of this study. I also

wish to thank Dr. L. R. Dice and Dr. F. C. Evans for their suggestions and criticisms in the preparation of this paper and Dr. W. J. Schull, Dr. F. E. Smith, and Mr. W. B. McIntosh for their valuable advice on statistical methods. Mr. P. J. Clark carefully read the manuscript and made many helpful suggestions. The study was conducted in the Laboratory of Vertebrate Biology.

MATERIALS AND METHODS

The stocks of mice used are as follows:

Peromyscus maniculatus blandus.—Taken in the vicinity of Tularosa, Otero County, New Mexico, in 1938, by W. F. and F. A. Blair.

P. maniculatus bairdi.—Taken in Washtenaw County, Michigan, in 1946.

P. polionotus leucocephalus.—Taken on Santa Rosa Island, Florida, in 1942, by W. F. Blair.

The animals were reared by the general methods described by Dice (1947). Only individuals from about two months to one year of age were used in the matings. Close inbreeding was avoided, and all matings in which the relationship was closer than first cousin were excluded. Each mating was examined for litters every day for a period of six months, starting three weeks after the mating was made. Those matings which produced one or more live-born young were considered to be fertile. The sterility of some mated females was later confirmed by the absence of placental scars in their uteri.

Formulae for the Measurement of Fertility and of Reproductive Isolation

Before an attempt is made to analyze or to interpret the experimental results, certain formulae needed for the measurement of fertility and of reproductive isolation are presented and, when necessary, a brief explanation is given of their derivation.

Only a fraction of laboratory-bred animals is fertile, as is probably true of field-living animals. This is undoubtedly due to many factors, involving both heredity and environment. It is first necessary, therefore, to make an estimate of the probability of an individual's being fertile. If b is the number of fertile matings among the total number, n_1 , of matings made, and if it is assumed that f , the probability of fertility, is equal and independent in the two sexes (this point will be discussed later), then the proportion of fertile matings is equal to the probability of a fertile female being mated with a fertile male, or $b/n_1 = f^2$. From this relationship the probability of an individual mouse being fertile is readily obtained as $f = \sqrt{b/n_1}$.

Therefore, the following relations are obtained:

Proportion of fertile matings,

$$f^2 = \frac{b}{n_1} \quad (1)$$

Probability of an individual being fertile,

$$f = \sqrt{\frac{b}{n_1}} \quad (2)$$

The standard deviation (S) of f is easily obtained by the maximum likelihood method (Mather, 1951), as follows:

$$S_f = \sqrt{\frac{1-f^2}{4n_1}} \quad (3)$$

Since it is not infrequent that certain males have been previously proved to be fertile, the probability of these males being fertile is unity, and as a result the proportion of fertile matings, c , among total matings, n_2 , involving these males should be equal to the proportion of fertile females among the total number of females used. That is:

Probability of an individual female being fertile,

$$f = \frac{c}{n_2} \quad (4)$$

The standard deviation in this case is:

$$S_f = \sqrt{\frac{f(1-f)}{n_2}} \quad (5)$$

Consequently, two different f values, f_1 and f_2 , may be calculated from the separate sets of data obtained from different mating types, that is, f_1 is calculated from one set of data gathered from matings in which the fertility of both parents is unknown, and f_2 is estimated from another set of data gathered from matings in which the fertility of only the female parent is unknown. The significance of the difference of these two f values from zero can be tested by the standard deviation method. Thus, if the standard deviation of f_1 is S_{f_1} , and that of f_2 is S_{f_2} , and if the two sets of data are independent, then the standard deviation of $(f_1 \pm f_2)$ is:

$$S_{f_1 \pm f_2} = \sqrt{S_{f_1}^2 + S_{f_2}^2},$$

where S_{f_1} and S_{f_2} are shown in equations (3) and (5), respectively.

Hence the difference between $(f_1 - f_2)$ and zero in terms of the standard deviation of $(f_1 - f_2)$ is:

$$t = \frac{f_1 - f_2}{\sqrt{S_{f_1}^2 + S_{f_2}^2}}$$

and the probability associated with any t value can be obtained from a table of the normal distribution.

If these two f values differ statistically as tested by the standard deviation method, then let f' be the probability of a female being fertile, and substitute it in equation (1):

$$ff' = \frac{b}{n_1}$$

or

$$f = \frac{b}{f' n_1} \quad (6)$$

Since b , n_1 , and f' are known, equation (6) is readily solved for f , which is the probability of an individual male being fertile. But f and f' are not obtained independently and there are no means of testing whether the difference between f and f' is significantly different from zero. When these two f values are known, all the other equations with the exception of (7) and (8) can be readily solved.

In my experiments, except for the matings of *blandus*, two separate sets of data are not available. It is assumed that f and f' are of the same magnitude, and therefore the prime of f will be dropped in the following equations.

If there are two separate sets of data for any kind of matings, and if f can not be shown to differ in the two sets, it is logical to pool them to obtain a combined estimate of f by the maximum likelihood method.

Now, the joint probability, P , of obtaining precisely b fertile matings among n_1 matings when both parents are of unknown fertility and c fertile matings among n_2 matings when the male parent is known to be fertile is:

$$P\left(\frac{b}{n_1}, \frac{c}{n_2}\right) = \left[\binom{n_1}{b} (f^2)^b (1-f^2)^{n_1-b} \right] \left[\binom{n_2}{c} (f)^c (1-f)^{n_2-c} \right].$$

Intuitively, the best estimate of f is that value which maximizes P , that is, that estimate which makes most probable the observed sampling distribution. Now

$$\log P = \log K_1 + b \log f^2 + (n_1 - b) \log (1-f^2) + \log K_2 + c \log f + (n_2 - c) \log (1-f)$$

where

$$K_1 = \binom{n_1}{b}$$

$$K_2 = \binom{n_2}{c}$$

and both are constants depending on the coefficients of the likelihood term.

Maximizing this logarithm gives:

$$\frac{d \log P}{d f} = \frac{2b}{f} - 2 \frac{(n_1 - b)f}{1-f^2} + \frac{c}{f} - \frac{n_2 - c}{1-f} = 0 \quad (7)$$

Equation (7) is a quadratic equation and is easily solved for f . The standard deviation of the maximum likelihood statistic f (Mather, 1951) is:

$$S_f = \sqrt{\frac{f(1-f^2)}{4n_1 f + n_2 (1+f)}} \quad (8)$$

When the probability of individual fertility of each parent stock is known, the reproductive isolation index of the intersubspecific and inter-specific crosses may be evaluated. Let f_a and f_b be the probabilities of

individual fertility for two parent stocks A and B, respectively, and f_{ab}^2 the proportion of fertile matings among the total cross matings of the type female stock A times male stock B. If no disturbing factors are involved, such as psychologic or gametic isolation, one would expect:

$$f_{ab}^2 = f_a f_b .$$

The reproductive isolation index may now be defined as the ratio of the difference between the product of the two probabilities of individual fertility for two parent stocks and the observed proportion of fertile matings among the total cross matings to the sum of these two quantities. Therefore, if one measures the reproductive isolation between the female stock A and male stock B, the formula is:

$$I_{ab} = \frac{f_a f_b - f_{ab}^2}{f_a f_b + f_{ab}^2} . \quad (9)$$

Here $f_a f_b$ is used in both the numerator and the denominator to account for the effect of captivity in the laboratory. Although the laboratory conditions may be made as suitable to the animals as possible, they are never perfect, and the fertility of one stock might be more influenced by captivity than that of the other. One would, therefore, expect the fertility of cross matings to be affected in varying degrees according to the parent stocks used. In other words, cross fertility is a function of the probabilities of individual fertility in the two parent stocks. If, therefore, the proportion of fertile matings in an outcross is equal to the product of the two probabilities of individual fertility of two parent stocks, the reproductive isolation index is equal to zero. In all other cases the values may fluctuate in either direction, but it is easily seen that the values are bounded between -1.0 and +1.0. If all cross matings are sterile, the value of the index is +1.0. If negative values of the index are obtained, cross mating occurs in preference to matings between individuals of the same stocks.

Similarly, to measure the reproductive isolation of the reciprocal cross, i.e., the mating between female stock B and male stock A, the equation is:

$$I_{ba} = \frac{f_b f_a - f_{ba}^2}{f_b f_a + f_{ba}^2} . \quad (10)$$

Finally, these two types of reciprocal matings may be combined, and their joint reproductive isolation index, $I_{(ab)}$, measured. The formula is:

$$I_{(ab)} = \frac{f_a f_b - f_{(ab)}^2}{f_a f_b + f_{(ab)}^2} \quad (11)$$

where $f_{(ab)}^2$ is the proportion of fertile matings in both reciprocal crosses of stocks A x B. In using this formula, it is desirable that the number of matings in the two reciprocal crosses be equal. The effect on the reproductive isolation index of marked departures from equality in the

number of matings in the two reciprocal crosses has not been explored.

The statistical significance of departures of the observed values from the expected values, that is, $f_a f_b$, can readily be tested by chi-square as shown by Table I.

TABLE I
Scheme for Chi-square Test

Mating	Fertile Matings		Sterile Matings	Chi-square	d. f.
Cross between female stock A and male stock B	Observed	b	$n_1 - b$	$\frac{(b - n_1 f_a f_b)^2}{n_1 f_a f_b (1 - f_a f_b)}$	1
	Expected	$n_1 f_a f_b$	$n_1 (1 - f_a f_b)$		
Cross between female stock B and male stock A	Observed	c	$n_2 - c$	$\frac{(c - n_2 f_b f_a)^2}{n_2 f_b f_a (1 - f_b f_a)}$	1
	Expected	$n_2 f_b f_a$	$n_2 (1 - f_b f_a)$		
Both reciprocal crosses	Observed	b + c	$n_1 + n_2 - b - c$	$\frac{[(b+c) - (n_1+n_2)f_a f_b]^2}{(n_1+n_2)f_a f_b (1 - f_a f_b)}$	1
	Expected	$(n_1+n_2)f_a f_b$	$(n_1+n_2)(1 - f_a f_b)$		

Analysis of Chi-square

Item	Chi-square	d. f.
Total	Sum of two χ^2 's for two reciprocal crosses	2
Deviation	χ^2 from the item of both reciprocal crosses	1
Heterogeneity	Total χ^2 minus deviation χ^2	1

From Table I two kinds of chi-square are obtained for comparison. One is the total chi-square, which is obtained from the sum of two chi-squares for two reciprocal crosses, and the other is the deviation chi-square, which is obtained directly from the item of both reciprocal crosses. The difference between these two chi-squares is the heterogeneity chi-square, which is used to test the agreement of the two reciprocal crosses. The analysis is presented in tabular form. Details will be given later in the numerical analysis.

ANALYSIS OF DATA

The results of breeding within each of the three parent stocks, namely, *P. p. leucocephalus*, *P. m. blandus*, and *P. m. bairdi*, are summarized in Table II.

In these parent stocks the probability of individual fertility is highest

in blandus. The probability value of blandus is 0.94, that of bairdi is 0.69, and that of leucocephalus is low, only 0.56. The wide difference in the probability of individual fertility among these three parent stocks suggests that, among other things, the laboratory conditions were more favorable to the blandus stock than to the others.

In the case of blandus, data from two separate sets of matings are available, namely, one set from matings in which the fertility of both sexes is unknown, and another from the matings in which the fertility of the males is known from previous matings. The fertility in these two sets of matings, as measured by their f values, is not significantly different statistically ($t = 0.94$, $P = 0.40-0.30$). The best estimation of the f value may be obtained by applying the maximum likelihood method (equation 7), with the following results:

$$\frac{2(28)}{f} - \frac{2(4)f}{1-f^2} + \frac{18}{f} - \frac{3}{1-f} = 0.$$

The solution of this equation gives an f value of 0.91, and substitution of this f value in equation (8) gives $S_f = 0.03$. Since this probability of individual fertility and its standard deviation are obtained from the combined estimation of two sets of data, these values will be used for blandus in later calculations.

TABLE III
Reproductive Isolation Index of Certain Intersubspecific
and Interspecific Crosses in *Peromyscus*

Mating	Fertile Matings	Sterile Matings	Total	Proportion of Fertile Matings Among Total (f^2)	Reproductive Isolation Index (I)
♀ <i>P. p. leucocephalus</i> x ♂ <i>P. m. blandus</i>	10	54	64	0.16	0.52
♀ <i>P. m. blandus</i> x ♂ <i>P. p. leucocephalus</i>	10	21	31	0.32	0.23
Both reciprocal crosses	20	75	95	0.21	0.42
♀ <i>P. m. bairdi</i> x ♂ <i>P. m. blandus</i>	14	33	47	0.30	0.35
♀ <i>P. m. blandus</i> x ♂ <i>P. m. bairdi</i>	34	11	45	0.76	-0.10
Both reciprocal crosses	48	44	92	0.52	0.09

Since the probability of individual fertility in the three parent stocks is known, equations (9), (10), and (11) are used to derive reproductive isolation indices for the intersubspecific and interspecific crosses. Their results are shown in Table III, and their chi-square values are shown in Tables IV and V, respectively.

TABLE IV
Chi-square Test for Reproductive Isolation in Crosses
between *P. p. leucocephalus* and *P. m. blandus*

Mating	Fertile Matings	Sterile Matings	Total	χ^2	d. f.	P
♀ <i>P. p. leucocephalus</i> x ♂ <i>P. m. blandus</i>	Observed 10 Expected 32.61	54 31.39	64 64	31.97	1	<0.01
♀ <i>P. m. blandus</i> x ♂ <i>P. p. leucocephalus</i>	Observed 10 Expected 15.80	21 15.20	31 31	4.34	1	0.05-0.02
Both reciprocal crosses	Observed 20	75	95	33.99	1	<0.01
	Expected 48.41	46.59	95			

Analysis of Chi-square

Item	χ^2	d. f.	P
Total	36.31	2	<0.01
Deviation	33.99	1	<0.01
Heterogeneity	2.32	1	0.20-0.10

The reproductive isolation index between ♀ *leucocephalus* and ♂ *blandus* is quite high. Its value, 0.52, is statistically significant. The reproductive isolation index of the reciprocal cross is only 0.23, which is barely significant ($P = 0.05-0.02$). The joint reproductive isolation index of the two reciprocal crosses is 0.42, which, again, is statistically significant. Finally, the heterogeneity test shows that the data from these two reciprocal crosses are homogeneous.

The over-all picture given by these figures indicates that a certain amount of reproductive isolation has developed between *leucocephalus* and *blandus*, but that the degree of reproductive isolation is somewhat different in the reciprocal crosses. These two species occupy different geographic areas and ecologic niches with no naturally interbreeding intermediates. Since there is no chance for gene exchange between them in nature, genetic differences presumably have accumulated, and gradually reproductive isolation has been built up. In addition, *leucocephalus* is smaller in body size. When this species is used as the female parent in the cross, difficulty frequently arises in parturition (Watson, 1942).

It has been found that over half of the leucocephalus mothers died with their fetuses, but such high mortality does not occur if the larger blandus is used as the female parent. This is one of the facts which give an explanation of the difference of reproductive isolation indices between these two reciprocal crosses.

Reproductive isolation between blandus and bairdi presents different statistics. The reproductive isolation index of the cross between ♀ bairdi and ♂ blandus is 0.35, which is statistically significant, and that of the reciprocal cross is -0.10, statistically nonsignificant, whereas

TABLE V
Chi-square Test for Reproductive Isolation in Crosses
between *P. m. blandus* and *P. m. bairdi*

Mating	Fertile Matings	Sterile Matings	Total	χ^2	d. f.	P
♀ <i>P. m. bairdi</i> x ♂ <i>P. m. blandus</i>	Observed 14 Expected 29.51	33 17.49	47 47	21.91	1	<0.01
♀ <i>P. m. blandus</i> x ♂ <i>P. m. bairdi</i>	Observed 34 Expected 28.26	11 16.74	45 45	3.13	1	0.10-0.05
Both reciprocal crosses	Observed 48	44	92	4.44	1	0.05-0.02
	Expected 57.77	34.23	92			

Analysis of Chi-square

Item	χ^2	d. f.	P
Total	25.04	2	<0.01
Deviation	4.44	1	0.05-0.02
Heterogeneity	20.60	1	<0.01

the joint reproductive isolation index of the two reciprocal crosses is 0.09, barely significant ($P = 0.05-0.02$). The chi-square test for heterogeneity shows that the fertility is different in the two reciprocal crosses.

These findings indicate that some reproductive isolation between bairdi and blandus probably exists, but that the joint reproductive isolation in the two reciprocal crosses is negligible (0.09). If one inspects the geographic distribution patterns of these two subspecies, it is found that intermediate interbreeding forms exist (Osgood, 1909). These facts coincide with the Laboratory findings that reproductive isolation between these two subspecies is negligible. But there is a significant heterogeneity, i.e., the fertility of the two reciprocal crosses is not the same. The question arises, therefore, of why a particular cross

between *bairdi* and *blandus* shows reproductive isolation but the reciprocal does not. This is a difficult question to answer. Nevertheless, a clue may be found in the difference in individual fertility of the two parent stocks. It seems likely that the female may play a more important role in reproductive isolation than the male. To demonstrate whether this is true or not will require further experiments.

SUMMARY

Several simple formulae are presented for the measurement of fertility within and between parent stocks. The probability of individual fertility (f) of a parent stock, on which all other calculations depend, is equal to the square root of the proportion of b fertile matings among the total n_1 , when the fertility of both sexes is unknown, or

$$f = \sqrt{\frac{b}{n_1}},$$

and is equal to the proportion of c fertile matings among the total n_2 , when one sex is known to be fertile from a previous mating, or

$$f = \frac{c}{n_2}.$$

The reproductive isolation index is used to measure the reproductive isolation quantitatively and is defined as the ratio of $(f_a f_b - f_{ab}^2)$, the difference between the product of the two probabilities of individual fertility for two parent stocks and the observed proportion of fertile matings among the total cross matings, to $(f_a f_b + f_{ab}^2)$, the sum of these two quantities, or

$$I_{ab} = \frac{f_a f_b - f_{ab}^2}{f_a f_b + f_{ab}^2}.$$

The statistical significance of the departures of the observed values from the expected values is ascertained by the chi-square test.

The probabilities of individual fertility thus obtained are quite different in the three parent stocks studied. It is highest in *Peromyscus maniculatus blandus* (0.91), lower in *P. m. bairdi* (0.69), and lowest in *P. polionotus leucocephalus* (0.56). This probably means that, among other things, the laboratory conditions were more favorable to *blandus* than to *leucocephalus*.

The reproductive isolation indices of the cross *leucocephalus* x *blandus* and of its reciprocal cross are rather high (0.52 and 0.23), hence their joint reproductive isolation index is also high (0.42) and statistically significant. This shows that some reproductive isolation exists between these two species. The reproductive isolation indices of the cross *bairdi* x *blandus* and its reciprocal are either low or negative (0.35 and -0.10); in turn, the joint reproductive isolation index is very small (0.09) and statistically barely significant. This indicates that the reproductive isolation between these two subspecies is negligible.

LITERATURE CITED

DICE, L. R.

1933 Fertility relationships between some of the species and subspecies of mice in the genus *Peromyscus*. *Journ. Mammalogy*, 14: 298-305.

1947 Deer mice: methods for the rearing of *Peromyscus*. In: *The UFAW handbook on the care and management of laboratory animals*. London: Bailliere, Tindall & Cox. Pp. 171-77.

MATHER, K.

1951 *The measurement of linkage in heredity*. 2d ed.; London: Methuen & Co.

OSGOOD, W. H.

1909 Revision of the mice of the American genus *Peromyscus*. U. S. Dept. Agric., *N. Amer. Fauna*, 28: 1-285.

WATSON, M. L.

1942 Hybridization experiments between *Peromyscus polionotus* and *Peromyscus maniculatus*. *Journ. Mammalogy*, 23: 315-16.

Copies of this contribution may be purchased from the University of Michigan Press, Ann Arbor, Michigan. Price, \$0.20.